

Figure 1.
Cloning vectors for the expression of Udp and PNP enzymes

Plasmid pUC18: 5' sequence of *lacZ* gene

RBS	$\xrightarrow{\text{EcoRI}}$ <u>AGGAGAACAGCT</u> ATG ACC ATG ATT ACG AAT TCG AGC TCG <u>GTA</u> CCC GGG GAT CCT CTA GAG TGC ACC TGC AGG CAT GCA AGC TRG	<u>thr</u> met <u>ile</u> <u>thr</u> <u>asn</u> <u>ser</u> <u>ser</u> <u>ser</u> <u>val</u> <u>pro</u> <u>gly</u> <u>asp</u> <u>pro</u> <u>leu</u> <u>glu</u> <u>ser</u> <u>thr</u> <u>cys</u> <u>arg</u> <u>his</u> <u>ala</u> <u>ser</u> <u>leu</u>	<u>Sali</u> <u>KpnI</u> <u>HindIII</u> <u>SphI</u>
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plasmid pGM678 and pGM707: sequence of *lacZ-deoD* fused genes

plasmid pGM679 and pGM708: sequence of *lacZ*-*ucp* fused genes

palmsid PGM712 e PGM716: 5' and 3' sequence of *deob* gene

Sali/NheI RBS EcoRI Sali SphI
GTGCACTAGGAGGAATTCTTC ATG GCT ACC CCA..... TCG GCG TAA AGAGTAAGTCGACCTGAGGCATGCAA
GTGCACTAGGAGGAATTCTTC met ala thr pro..... trp ala stop

Figure 2. 5' and 3' sequences of *udp e deoD* genes cloned in plasmid pUC18. Restriction sites of different constructs are underlined; the ribosome binding site (RBS) is reported in bold. The bases of nucleotide sequence of *udp* and *deoD* genes and the amino acid residues of PNP and UDP proteins are reported in italics.

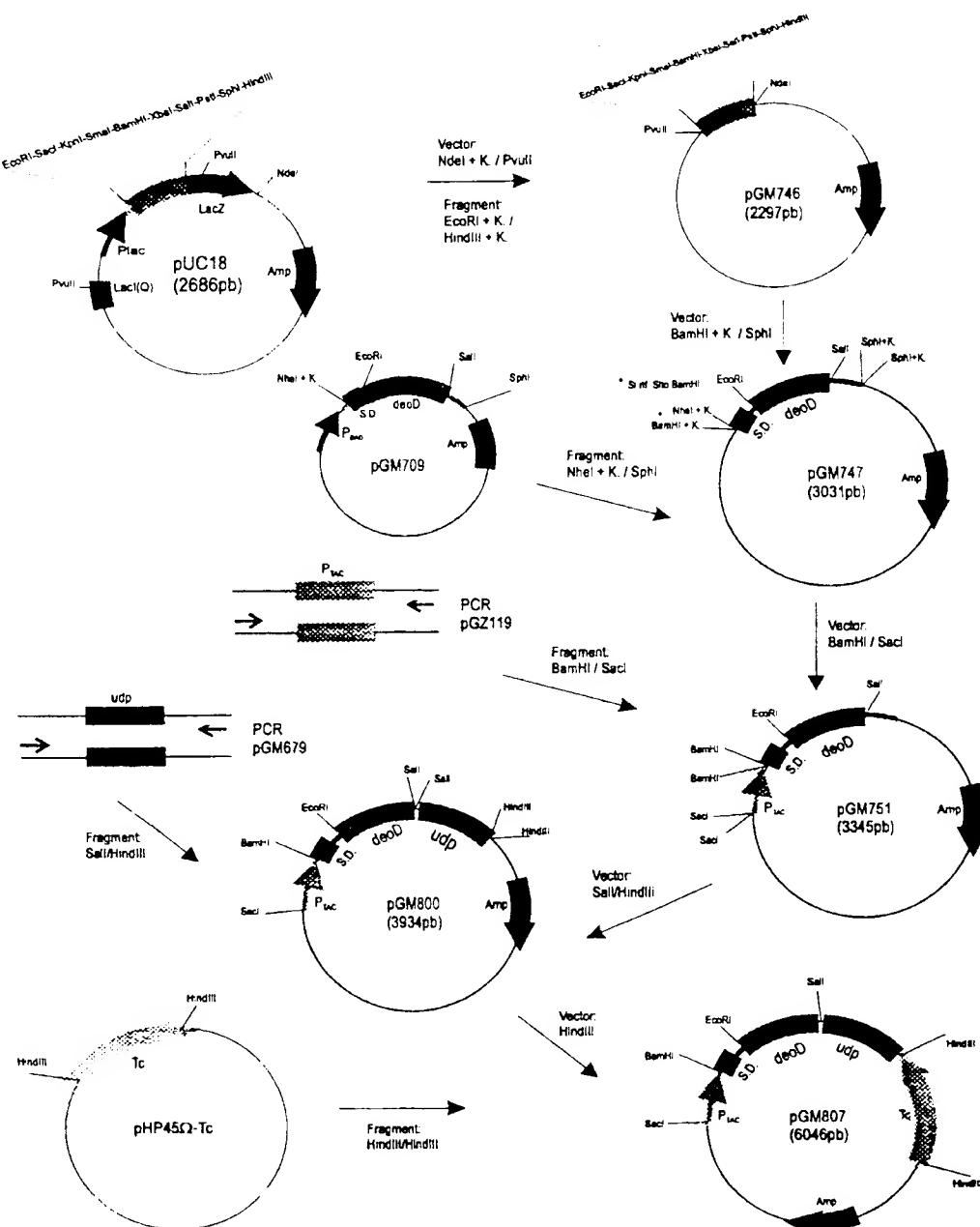


Figure 3.
Construction of cloning vectors for the expression of Udp and PNP enzymes

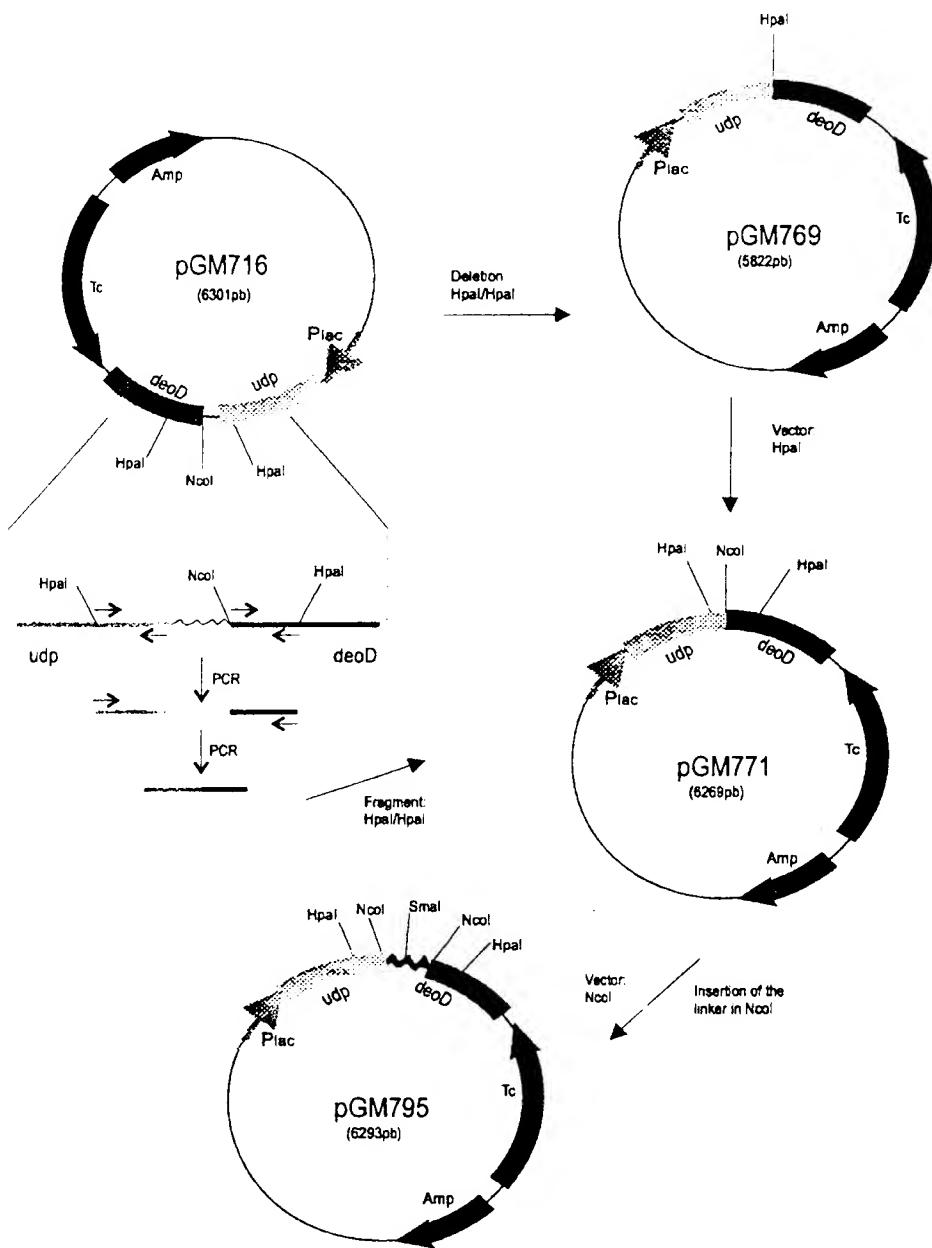


Figure 4.

Construction of cloning vectors for the expression of UDP-(L)-PNP enzymes.

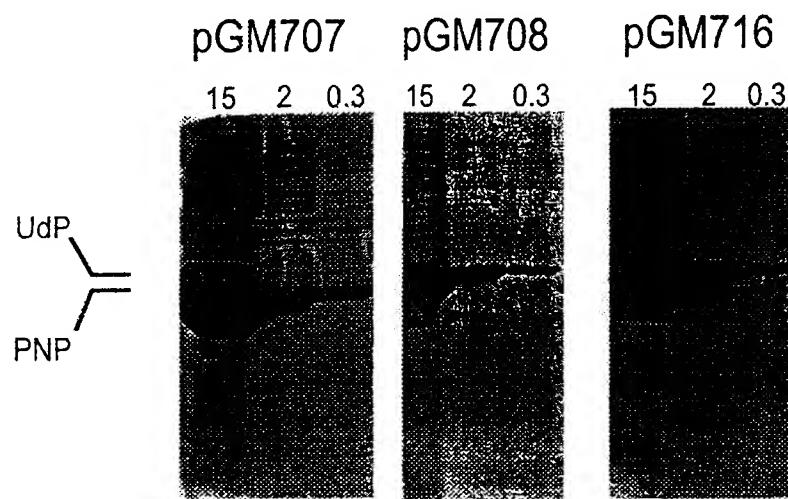


Figure 5.

Expression of PNP and UdP in recombinant *E. Coli* strains. Gel electrophoresis (SDS-PAGE) of total protein extracts from strains MG1655/pGM707, MG1655/pGM708 and MG1655/pGM716 grown over night in LD medium suplemented with 12.5 mg/liter of tetracycline. Lanes 15, 2 and 0.3 correspond to protein extracted from 15, 2 and 0.3 ml of bacterial culture.